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OOCYST EXCRETION PATTERN OF THREE INTESTINAL *EIMERIA* SPECIES IN FEMALE RABBITS

PAPESCHI C.*, FICHI G.†, PERRUCCI S.†

*Dipartimento di Ecologia e Scienze Biologiche, Università degli Studi della Tuscia, Largo dell'Università, 01100 Viterbo, Italy.

†Dipartimento di Scienze Veterinarie, Università di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy.

Abstract: The dynamic change in faecal *Eimeria* oocyst excretion was evaluated in 10 naturally infected female rabbits, starting from their weaning at 33 d of age until about 1 mo after their second parturition. Faecal samples collected from examined animals were quali-quantitatively analysed to evaluate presence and number of *Eimeria* oocysts. In addition, isolated *Eimeria* oocysts were identified at the species level following sporulation. Animals were found to be infected by *Eimeria perforans, Eimeria exigua* and *Eimeria magna* and shed *Eimeria* oocysts after weaning and after parturition. In particular, at 33 d of age all female rabbits examined were negative, while the discharge of *Eimeria* oocysts started at 39th day of age and peaked between 46th and 53rd day of age. From 81-109 d of age until the first parturition and from 25 d of age of the litters born at the first parturition to the second parturition, all animals resulted negative. After parturition, *Eimeria* oocyst output occurred from 6th to 12th day after the first parturition and from 7th to 13th day after the second parturition, while a second period of oocyst excretion was observed from 18th to 24th day after both parturitions. These findings may indicate the existence of a relationship between the periparturient phase and *Eimeria* oocyst output and suggest an important role of the mothers in transmission of the infection to their litters.

Key Words: *Eimeria*, oocyst excretion pattern, weaning, periparturient phase, female rabbits.

INTRODUCTION

Coccidiosis is a major parasitic disease of the rabbit caused by protozoa of the genus *Eimeria* (Apicomplexa: Eimeridae) that affects mainly young rabbits after weaning (Drouet-Viard *et al.*, 1997; Pakandl and Hlásková, 2007) and can be responsible for important economic losses in rabbit farms (Bhat *et al.*, 1996; Taylor *et al.*, 2007; Li *et al.*, 2010).

Coccidial infection is initiated by oral ingestion of sporulated oocysts by the susceptible host and the infection can lead to clinical coccidiosis primarily in kits, whereas adults are mostly healthy carriers (Bhat *et al.*, 1996; Coudert *et al.*, 2000). According to their pathogenicity, species responsible for rabbit intestinal coccidiosis can be classified into 4 types: non pathogenic (*Eimeria coecicola*), slightly pathogenic (*Eimeria exigua*, *Eimeria perforans*, *Eimeria vejdovskyi*), mildly pathogenic or pathogenic (*Eimeria irresidua*, *Eimeria magna*, *Eimeria media*, *Eimeria piriformis*) and highly pathogenic (*Eimeria intestinalis*, *Eimeria flavescens*) (Coudert *et al.*, 1995; Taylor *et al.*, 2007; Pakandl, 2009). Clinical signs of the disease include failure to gain weight, poor feed conversion, growth retardation, diarrhoea, anaemia and possibly death (Coudert *et al.*, 1995; Bhat *et al.*, 1996; Taylor *et al.*, 2007; Pakandl, 2009).

Previous studies have shown that rabbits younger than 19-21 d cannot be infected by coccidia (Pakandl and Hlásková, 2007; Paklandl, 2009) even when experimental animals are kept coccidia-free for several generations (Pakandl *et al.*, 2008), while kits are more frequently infected from around 5-6 wk of age (Drouet-Viard *et al.*, 1997; Grès *et al.*, 2003) to around 3 mo of age (Gomez-Bautista *et al.*, 1987). Concerning oocyst shedding pattern in female rabbits used for reproduction, it was previously shown (Połozowski, 1993) that 2 peaks occurred after parturition: the first one in the perinatal period and the second in the period preceding weaning of the litters. The present study was designed to evaluate the dynamic change of *Eimeria* oocyst excretion in naturally infected female rabbits from their weaning until the weaning of the litters born from their second parturition.

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MATERIALS AND METHODS

Animals

The study was conducted in 2003 on 10 "Leprino di Viterbo" breed (ANCI, 2012) weaned female rabbits (mean weight 0.803±0.062 kg at the beginning of the experiment) from the same farm. The experimental period was from females weaning, at 33 d of age, until the weaning of the litters born from their second parturition. Animals were reared in individual metallic cages (150×100×76 cm, length×width×high) with a wire mesh floor and fed a commercial laboratory pelleted feed free of anticoccidial drugs. Water was distributed *ad libitum*. On a daily basis, faeces and urine were removed, cages were cleaned and water and food containers were washed and refilled. Reproduction was natural and the 2 parturitions occurred in late spring (in May) and late autumn (in November). The nest (46×27×25 cm) was placed in the cage 3 d before the presumed day of parturition and removed at 20 d of age of the litter. Weaning of the litters occurred at 33 d of age.

Throughout the study period, animals were checked daily to observe clinical signs, such as diarrhoea, indicating a clinical form of coccidiosis and their weight was recorded weekly.

All experiments were carried out in accordance with the guidelines set by Italian law on the use of animals in research.

Sampling

Faecal samples were collected from each examined animal by means of plastic sheets placed under the wire mesh of the cage floor. From the beginning of the study at 33 d of age until the first parturition at about 165 d of age, and from the weaning of the litters born from the first parturition until the second parturition at about 343 d of age, samples were collected twice a week at 8.00 a.m. Faecal samples were instead collected daily at 8.00 a.m. during the period starting from the parturition of each female rabbit until weaning of the respective litter, i.e. for 33 consecutive days after each parturition. Individual faecal samples were also collected daily from all the males used for mating in the 2 wk before they were placed in the females' cages.

Parasitological Analysis.

Samples were quali-quantitatively analysed by flotation test using a low density solution (specific gravity 1.2) (Coudert *et al*., 1995) and by a modified McMaster method with a sensitivity of 20 oocysts/gram of faeces (OPG) (Permin and Hansen, 1998), to assess the presence and number of *Eimeria* oocysts.

To identify isolated *Eimeria* species, oocysts from faecal samples were allowed to sporulate by suspending them in 2.5% potassium dichromate ($K_2Cr_2O_7$) in Petri dishes. Dishes were checked daily until the sporulation of the oocysts. Oocysts, sporocysts, sporozoites and other structures were microscopically observed by immersion oil (1000×) and measured by means of an eye-piece micrometer. For the identification of isolated *Eimeria* species, descriptions given by several authors (Levine and Ivens, 1972; Pellerdy, 1974; Eckert *et al*., 1995; Grès *et al*., 2002; Taylor *et al*., 2007) were used. The frequency of each species in each culture was determined in 50-100 mature oocysts.

Statistical Analysis.

Data obtained from the evaluation of the OPG number were statistically elaborated with the analysis of variance and with the Test of Student of Newman-Keuls for multiple comparisons with $P<0.05$ significance (Glantz, 2003a), using McGraw-Hill software (Glantz, 2003b).

RESULTS

From parasitological analysis at weaning (33 d of age), all female rabbits examined were negative for *Eimeria* oocysts. Oocysts shedding started at 39 d of age in 3/10 of examined rabbits, while at 42 d of age all animals (10/10) were shedding *Eimeria* oocysts (Figure 1). Statistically, significantly ($P<0.05$) higher oocyst counts (2406±41 OPG, mean ± standard deviation of the period) were observed between 49th and 53rd days of age of examined animals (Figure 2). Compared to previous days, oocysts number significantly decreased ($P<0.05$) from 56th to 60th (1010±191 OPG) and

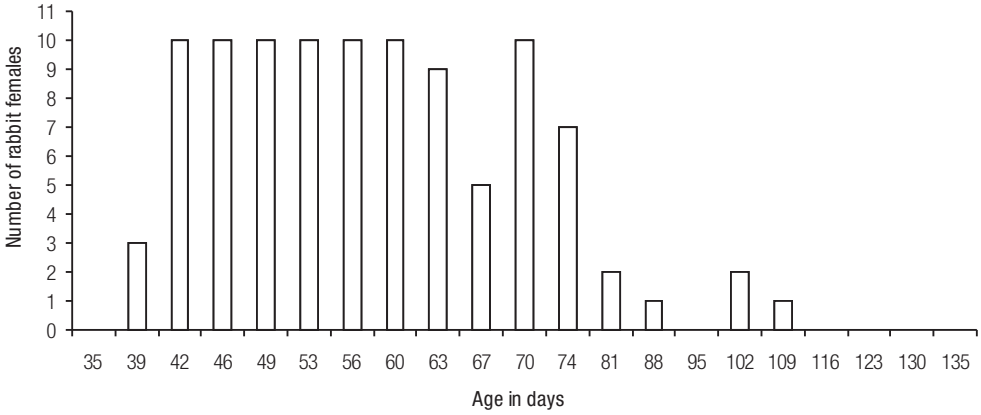

Figure 1: Number of rabbit females found to shed *Eimeria perforans, Eimeria exigua* and *Eimeria magna* oocysts from weaning at 33 d of age to mating at 135 d of age.

from 63rd to 109th (122±216 OPG) day of age. In this latter period, a decrease in the number of positive rabbits was also observed (Figure 2). Afterwards, all animals were negative till mating (Figure 2), at the age of 135 d, and also from mating to the first parturition, at about 165 d of age, except for 2 animals that excreted 200 *Eimeria* OPG at 143 d and at 150 d of age, respectively.

After the first parturition, all the mothers were negative till the 5th day *post-partum*, while faecal *Eimeria* oocysts appeared between the 6th and the 12th (458±245 OPG) day. Oocyst excretion stopped between the 13th and the 17th day and started again from the 18th till the 24th (331±208 OPG) day after parturition (Figure 3). From 25 d of age of the litters born at the first parturition to the second parturition, all examined female rabbits were negative.

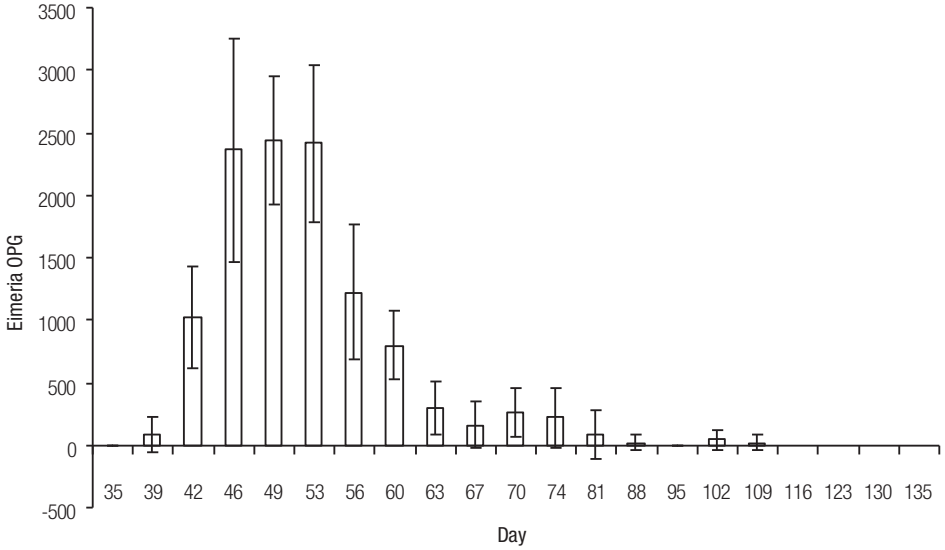

Figure 2: Mean output of *Eimeria perforans, Eimeria exigua* and *Eimeria magna* oocysts/gram of faeces (OPG) observed in 10 rabbit females from weaning at 33 d of age to mating at 135 d of age.

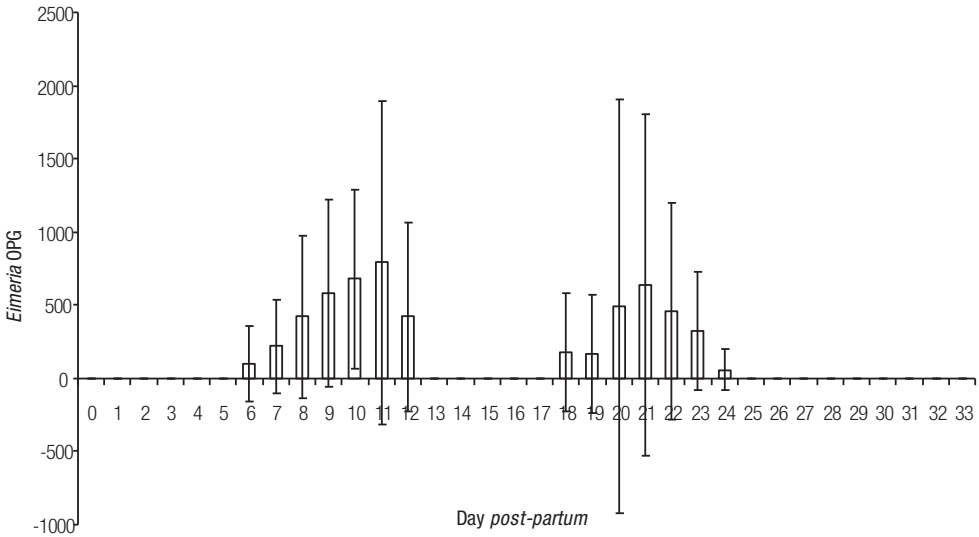

Figure 3: Mean output of *Eimeria perforans, Eimeria exigua* and *Eimeria magna* oocysts/gram of faeces (OPG) observed in 10 rabbit females from the first parturition in May 2003 to the weaning of their litter.

A similar trend, characterised by 2 periods of oocyst emission from the parturition to the weaning of the litters, was observed in all female rabbits also after the second parturition. More precisely, the examined female rabbits shed faecal oocysts from 7th to 13th day *post-partum* (Figure 4), with a significantly higher emission (P<0.05) between the 9th to the 11th day (533±50 OPG), and from the 18th till the 24th day (277±167 OPG) after the parturition. Oocyst output stopped from 25th day *post-partum* till the weaning of the litters (Figure 4).

Statistically, highly significant differences (P<0.01) resulted from the multiple comparison of the mean OPG counts observed after weaning and after the first and second parturitions. More in particular, the mean OPG count recorded in the juvenile period (673±143 OPG) was significantly higher (P<0.05) compared to the mean OPG count observed both after the first (459±273 OPG) and after the second (346±145 OPG) parturition, while no differences emerged from the comparison of these 2 latter values. All faecal samples from the males used for mating were negative for *Eimeria* oocysts.

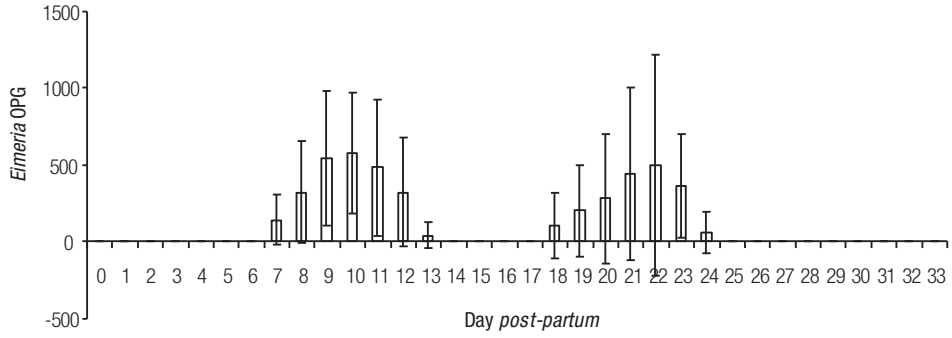

Figure 4: Mean output of *Eimeria perforans, Eimeria exigua* and *Eimeria magna* oocysts/gram of faeces (OPG) observed in 10 rabbit females from the second parturition in November 2003 to the weaning of their litter.

From microscopic analysis of sporulated oocysts, *Eimeria perforans, Eimeria exigua* and *Eimeria magna* were the species identified throughout the study period and in all examined female rabbits. In mean, *E. perforans* (61%) oocysts dominated, while *E. magna* (32%) and *E. exigua* (7%) oocysts were less represented.

Through all the study, animals did not show clinical signs of coccidiosis and their weight was in normal range (from 3.6 to 4.2 kg at the end of the study).

DISCUSSION

In the 10 rabbit females observed in this study from their weaning, at 33 d of age, till the weaning of the litters born from their second parturition, quali-quantitative evaluation of faecal *Eimeria* oocyst shedding pattern showed the occurrence of *E. perforans, E. magna* and *E. exigua* oocyst emission after weaning and after each parturition. In particular, at 33 d of age all examined animals were found negative while oocyst shedding started from the 39th day of age, peaked between 46th and 53rd day of age and stopped at 81-109 d of age. These data confirm the outcomes of previous studies reporting that *Eimeria* infections occur mainly in kits just after weaning (Drouet-Viard *et al.*, 1997; Pakandl and Hlásková, 2007).

In addition, the female rabbits examined showed 2 periods of oocyst excretion after parturition: the first in the perinatal period and the second in the period just preceding weaning of the litter. In particular, the first excretion of *Eimeria* oocysts appeared from 6th to 12th d after the first parturition, in late spring, and from 7th to 13th d after the second parturition, in late autumn, while the second emission period was observed from 18th to 24th d after both the first and the second parturitions. Indeed, in this study the trend of oocyst emission was very similar after both parturitions in terms of oocyst count, length of the oocyst shedding period (number of days) after parturition and the number of shedding animals. All these findings seem to confirm previously reported data (Połozowski, 1993) concerning a relationship between *Eimeria* oocyst output and the periparturient phase.

Although in previous studies (Gallazzi, 1977; Nosal *et al.*, 2006) cyclical variations in the excretion of intestinal *Eimeria* oocysts in rabbits appeared to be mostly affected by the seasonal period, no differences emerged in the present study from the comparison of data observed in examined female rabbits in the 2 different periods of the year following the first (in May) and second (in November) parturitions. Therefore, other factors that occur during the periparturient period should be responsible for the faecal oocysts shedding observed in examined female rabbits.

A periparturient rise of *Eimeria* spp. oocyst faecal count was also observed previously in other mammalian species (Faber *et al.*, 2002; Taylor *et al.*, 2007; Bruhn *et al.*, 2011; Turner *et al.*, 2012). Moreover, the periparturient increase in the emission of (oo)cysts was also reported for other intestinal protozoa, such as *Cryptosporidium parvum* and *Giardia duodenalis* in infected sheep and goats (Xiao *et al.*, 1994; Ortega-Mora *et al.*, 1999; Castro-Hermida *et al.*, 2005). In wild rabbits, the periparturient increase in the emission of *Trichostrongylus retortaeformis* eggs was evidenced (Hobbs *et al.*, 1999). According to Marai *et al.* (2010), stress conditions and hormonal changes occurring in rabbits during pregnancy, parturition or suckling periods may lead to lowered resistance to parasitic infections and heightened susceptibility in the dam. The increase in nutrient requirements during pregnancy and lactation is another factor that may have a relevant role in lowered resistance to parasitic infections, as demonstrated in sheep (Ortega-Mora *et al.*, 1999; Houdijk *et al.*, 2000; Kidane *et al.*, 2010). The possibility that the periparturient emission of *Eimeria* oocysts as observed in female rabbits herein examined may be the result of reinfections seems unlikely, mainly because no oocyst shedding was observed in any females examined in the periods from mating to first parturition and between the weaning of the litters born from the first parturition and the second parturition. In addition, female rabbits were reared in individual cages; cages were cleaned daily, food and water contamination with infective oocysts was prevented and the males used for mating were negative for *Eimeria* oocysts at coprological analysis. Consequently, the reactivation of a latent infection should represent the main factor responsible for the periparturient excretion of *Eimeria* oocysts observed in all female rabbits examined in this study.

Adult rabbits, which are usually symptomless carriers of coccidian infections, could serve as a potential source of infection for kits (Bhat *et al.*, 1996; Coudert *et al.*, 2000). Findings from this study suggest that mothers may play an important role in *Eimeria* transmission to the litters. In fact, from results obtained here, the 2 periods of oocyst emission observed in all examined female rabbits after parturition, i.e. from the 6th-7th to the 12nd-13th and from the

18th to the 24th day *post-partum*, could easily be responsible for infection of the litter, as from about 22 d of age suckling rabbits are susceptible to *Eimeria* infections (Pakandl and Hlásková, 2007; Pakandl, 2009). In addition, from around 22 d of age rabbits begin to consume solid feed other than milk (Pakandl, 2009) and the probability of *Eimeria* oocyst intake by kits is higher, while changes in the intestinal environment may enhance the ability of sporozoites to excyst from ingested oocysts, penetrate into the host cells and develop (Pakandl and Hlásková, 2007; Pakandl, 2009).

It is known that oocyst output often markedly varies among individual rabbits also under identical experimental conditions, probably because animals are influenced by individual response to stressors (Coudert, 1982; Pakandl, 2009). Therefore, this may explain why the number of *Eimeria* oocysts was highly variable among the subjects herein examined. On the other hand, mean oocyst number registered in the juvenile period of examined animals was significantly higher compared to those observed after the 2 parturitions. This may depend on the incomplete maturity of the immune system of kits and/or of the lack of a protective immune response deriving from previous infections (Gomez-Bautista *et al.*, 1987; Grès *et al.*, 2003; Gutierrez, 2003; Pakandl *et al.*, 2008). Indeed, in rabbits the development of an acquired resistance to *Eimeria* infections appears at about 3 mo of age (Gomez-Bautista *et al.*, 1987). Interestingly, at this age the discharge of *Eimeria* oocysts with faeces stopped in all female rabbits examined in this study.

Among *Eimeria* species responsible for natural coccidial infections in animals examined in this study, *E. perforans* and *E. magna* are included among the most frequent species reported in farm rabbits in Italy (Gallazzi, 1977; Vereecken *et al.*, 2012), while the prevalence of *E. exigua* is lower (Vereecken *et al.*, 2012). As for their pathogenicity, *E. perforans* and *E. exigua* are considered slightly pathogenic, while *E. magna* is considered mildly pathogenic to pathogenic (Coudert *et al.*, 1995; Bhat *et al.* 1996; Pakandl 2009). Overall, during the whole observation period and in all examined animals, *E. perforans, E. magna* and *E. exigua* oocyst numbers were always lower than 4000-5000 OPG, which is the number of *Eimeria* OPG considered advisable to apply only medical prophylaxis in rabbits infected by coccidia (Coudert *et al.*, 2000; Gutiérrez, 2003). Therefore, the low or mild pathogenicity and the low intensity of isolated *Eimeria* species could explain why clinical signs of coccidiosis were absent in all female rabbits examined in the present study and their weight was within normal range.

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